# INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT2004/000015

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/415 C12N9/90 A01H5/00 A01H5/08
A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 01/57224 A (HAUSE BETTINA ; STENZEL IRENE (DE); ZIEGLER JOERG (DE); INST PFLANZENB) 9 August 2001 (2001-08-09)<br>abstract<br>page 9, line 23 - page 10, line 3<br>page 11, line 1 - line 6<br>page 12, line 8 - line 25<br>claims 1-26<br>sequences 1,2<br>-----<br>-/-- | 1-4,<br>14-27 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 9 February 2005 | 08.03.05 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Mundel, C |

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT2004/000015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ZIEGLER JOERG ET AL: "Molecular cloning of allene oxide cyclase: The enzyme establishing the stereochemistry of octadecanoids and jasmonates" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 275, no. 25, 23 June 2000 (2000-06-23), pages 19132-19138, XP002182669 ISSN: 0021-9258 abstract figure 2 page 19134, paragraph 1 - paragraph 3 | 1-4, 14-20 |
| X | PERNAS MONICA ET AL: "A chestnut seed cystatin differentially effective against cysteine proteinases from closely related pests" PLANT MOLECULAR BIOLOGY, vol. 38, no. 6, December 1998 (1998-12), pages 1235-1242, XP002316939 ISSN: 0167-4412 abstract page 1235, left-hand column, line 1 - line 8 page 1235, right-hand column, line 3 - line 14 page 1236, left-hand column, line 5 - line 10 Materials and methods page 1236 figure 2 | 1,5-7, 14-27 |
| X | PERNAS M ET AL: "Biotic and abiotic stress can induce cystatin expression in chestnut" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 467, no. 2-3, 11 February 2000 (2000-02-11), pages 206-210, XP004260953 ISSN: 0014-5793 the whole document | 1,5-7, 14-27 |
| A | ARAI SOICHI ET AL: "Plant seed cystatins and their target enzymes of endogenous and exogenous origin" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 50, no. 22, 23 October 2002 (2002-10-23), pages 6612-6617, XP002316940 ISSN: 0021-8561 the whole document | 1,5-7, 14-27 |

-/--

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT2004/000015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 00/01804 A (UNILEVER N.V; UNILEVER PLC)<br>13 January 2000 (2000-01-13)<br>abstract<br>page 1, line 25 - line 27<br>sequence 13 | 1,8-10,<br>14-27 |
| X | CHYE M-L ET AL: "BETA-1,3-GLUCANASE IS HIGHLY-EXPRESSED IN LATICIFERS OF HEVEA BRASILIENSIS"<br>PLANT MOLECULAR BIOLOGY, NIJHOFF PUBLISHERS, DORDRECHT, NL,<br>vol. 29, no. 2, 1995, pages 397-402, XP009011522<br>ISSN: 0167-4412<br>abstract<br>page 397, left-hand column, line 5 - line 12<br>page 398, left-hand column, line 7 - right-hand column, line 20 | 1,8-10,<br>14-27 |
| A | BEFFA R ET AL: "Pathogenesis-related functions of plant beta-1,3-glucanases investigated by antisense transformation -- a review"<br>GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB,<br>vol. 179, no. 1,<br>7 November 1996 (1996-11-07), pages 97-103, XP004071970<br>ISSN: 0378-1119<br>the whole document | 1,8-10,<br>14-27 |
| X | GARCIA-CASADO GLORIA ET AL:<br>"Characterization of an apoplastic basic thaumatin-like protein from recalcitrant chestnut seeds"<br>PHYSIOLOGIA PLANTARUM,<br>vol. 110, no. 2, October 2000 (2000-10), pages 172-180, XP002304729<br>ISSN: 0031-9317<br>the whole document<br>abstract<br>page 172, right-hand column, line 7 - line 10<br>page 172, right-hand column, line 16 - page 173, left-hand column, line 5<br>Materials and methods<br>page 173<br>page 176, left-hand column, line 4 - right-hand column, line 2<br>page 178, left-hand column, line 22 - line 26 | 1,11-27 |

-/--

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT2004/000015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | STINTZI A ET AL: "PLANT 'PATHOGENESIS-RELATED' PROTEINS AND THEIR ROLE IN DEFENSE AGAINST PATHOGENS" BIOCHIMIE, MASSON, PARIS, FR, vol. 75, no. 8, 1993, pages 687-706, XP009006230 ISSN: 0300-9084 abstract the whole document | 1,11-27 |
| A | SCHAFLEITNER R ET AL: "Effect of virulent and hypovirulent Cryphonectria parasitica (Murr.) Barr on the intercellular pathogen related proteins and on total protein pattern of chestnut (Castanea sativa Mill.)" PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, vol. 51, no. 5, November 1997 (1997-11), pages 323-332, XP002304728 ISSN: 0885-5765 the whole document | 1-4, 14-27 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/PT2004/000015

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2-4 (completely) and 1, 14-27 (partially)

Nucleic acid sequence encoding regions for a Castanea sativa Allene Oxide Cyclase (AOC), chimeric genes, expresion cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

---

2. claims: 5-7 (completely) and 1, 14-27 (partially)

Nucleic acid sequence encoding regions for a Castanea sativa Cystatin, chimeric genes, expresion cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

---

3. claims: 8-10 (completely) and 1, 14-27 (partially)

Nucleic acid sequence encoding regions for a Castanea sativa beta-1,3-glucanase, chimeric genes, expresion cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

---

4. claims: 11-13 (completely) and 1, 14-27 (partially)

Nucleic acid sequence encoding regions for a Castanea sativa Thaumatin-like protein, chimeric genes, expresion cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

---

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/PT2004/000015

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 0157224 A | 09-08-2001 | DE 10004468 A1 | 23-08-2001 |
| | | AU 3023901 A | 14-08-2001 |
| | | WO 0157224 A2 | 09-08-2001 |
| | | EP 1252318 A2 | 30-10-2002 |
| | | JP 2004506406 T | 04-03-2004 |
| | | US 2004137590 A1 | 15-07-2004 |
| WO 0001804 A | 13-01-2000 | AU 4513599 A | 24-01-2000 |
| | | WO 0001804 A2 | 13-01-2000 |

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 02 AUG 2005
WIPO PCT

| Applicant's or agent's file reference 2004/01/PCT | **FOR FURTHER ACTION** | See Form PCT/IPEA/416 |
|---|---|---|
| International application No. PCT/PT2004/000015 | International filing date *(day/month/year)* 25.06.2004 | Priority date *(day/month/year)* 26.06.2003 |

International Patent Classification (IPC) or national classification and IPC
C12N15/82, C07K14/415, C12N9/90, A01H5/00, A01H5/08, A01H5/10

Applicant
CASTANIA SOCIEDADE AGROFLORESTAL, S.A. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 16 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☐ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☒ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 23.01.2005 | 01.08.2005 |

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer
Mundel, C
Telephone No. +49 89 2399- 7314

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/PT2004/000015

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   **Description, Pages**

   1-17 as originally filed

   **Sequence listings part of the description, Pages**

   1-12 received on 07.09.2004 with letter of 01.09.2004

   **Claims, Numbers**

   1-27 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

## Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   **see separate sheet**

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

## Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 5-13, 25-27 |
| | No: | Claims | 1-4, 14-24 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-27 |
| Industrial applicability (IA) | Yes: | Claims | 1-27 |
| | No: | Claims | |

2. Citations and explanations (Rule 70.7):

   **see separate sheet**

## Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

## Supplemental Box relating to Sequence Listing

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

**Re Item IV**

**Lack of unity of invention**

The separate inventions/groups of inventions are:

2-4 (completely) and 1, 14-27 (partially)
Nucleic acid sequence encoding regions for a Castanea sativa Allene Oxide Cyclase (AOC), chimeric genes, expression cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

5-7 (completely) and 1, 14-27 (partially)
Nucleic acid sequence encoding regions for a Castanea sativa Cystatin, chimeric genes, expression cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

8-10 (completely) and 1, 14-27 (partially)
Nucleic acid sequence encoding regions for a Castanea sativa beta-1,3-glucanase, chimeric genes, expression cassettes and replicable expression vector comprising said nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

11-13 (completely) and 1, 14-27 (partially)
Nucleic acid sequence encoding regions for a Castanea sativa Thaumatin-like protein, chimeric genes, expression cassettes and replicable expression vector comprising said

nucleic acids, plant genome, host cells and genetically modified plants comprising said chimeric genes and methods of improving the defence response signalling to the ink disease comprising the introduction into the plant of an expression cassette comprising said nucleic acid sequences.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The only common concept linking the different groups of inventions mentioned above can be considered as a Castanea sativa protein involved in pathogen resistance.

This common concept is not novel nor inventive for the following reasons :
The documents Pernas M. et al., Plant Molecular Biology 38 (1998) and Pernas M, et al., FEBS Letters 467 (2000) disclose a cystatin expressed in chestnut (Castanea sativa) after biotic or abiotic stress.
The document Schafleitner R. and Wilhelm E., Physiological and Molecular Plant Pathology (1997) 51 discloses the induction of a beta-1,3- glucanase in Castanea sativa after treatment with Cryphonectria parasitica.
The document Garcia-Casado G. et al., Physiologia plantarum 110 (2000) discloses the characterization of an apoplastic basic Thaumatin-like protein from chestnut (Castanea sativa).

In the light of this prior art, the International Search Authority fails to see what could be the inventive common concept linking the different groups of inventions mentioned above. Therefore, the present application is considered to lack unity in the sense of Rule 13.1 PCT and the different groups mentioned above are considered as independent inventions.

However, since the applicant has chosen to pay the search fees for all the additional inventions, the claims have been examined in their entirety.

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statements.**

**Invention I : Allene oxide cyclase.**

1. The present application refers to a nucleic acid encoding a Castanea sativa Allene Oxide Cyclase (nucleic acid : SEQ ID NO:1; polypeptide : SEQ ID NO:2), a chimeric gene comprising one or more such nucleic acids, an expression cassette, an expression vector, a plant genome or a host cell comprising such a chimeric gene, a genetically modified plant containing such a chimeric gene stably integrated in its genome and the progeny, fruits or seeds of such plant. The application also refers to methods of improving the defense of a plant comprising the introduction of an expression cassette according to the present application into the plant.

2. The following documents are referred to in this communication:

   D1 : WO 01/57224 A (HAUSE BETTINA ; STENZEL IRENE (DE); ZIEGLER JOERG (DE); INST PFLANZENB) 9 August 2001 (2001-08-09)

   D2 : ZIEGLER JOERG ET AL: "Molecular cloning of allene oxide cyclase: The enzyme establishing the stereochemistry of octadecanoids and jasmonates" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 275, no. 25, 23 June 2000 (2000-06-23), pages 19132-19138

3. **Lack of novelty; article 33(2) PCT.**

   3.1 The document D1 discloses a Lycopersicon esculentum allene oxide synthase (SEQ ID NO:2 of D1) having 100% identity with SEQ ID NO:2 of the present application over the entire length of SEQ ID NO:2. The corresponding nucleic acid (SEQ ID NO:1 of D1) presents 100% identity with the nucleic acid disclosed in SEQ ID NO:1 of the present application.

   D1 also discloses the transformation of host cells with said nucleic acid (p. 9,

line 23 to p. 10, line 6). The generation of transgenic plants is also disclosed (p. 11, lines 1-6). The use of the nucleic acid sequences for the generation of plants having enhanced pathogen resistance is suggested on p. 12 (second paragraph). Constructs (claims 7-9), host cells (claims 10-11), plant cells and tissues (claims 12-13) are claimed.

Therefore, the International Search Authority (ISA) considers that the subject-matter of claims 1-4 and 14-26 cannot be considered as novel over the teaching of D1 (article 33(2) PCT).

3.2 The document D2 discloses the same Lycopersicon esculentum allene oxide cyclase as D1 (the inventors of D1 are authors of D2).

The teaching of D2 differs of the teaching of D1 in that the use of the sequences for transforming plants is not discussed. However, transformed E. coli cells are disclosed (p. 19134, left hand column, Overexpression of AOC).

Therefore, the subject-matter of claims 1-4, 14-18 and 20 cannot be considered as novel over the teaching of D2 (article 33(2) PCT).

3.3 Since there is no clear definition of what a "chimeric gene" should be, each Lycopersicon esculentum cells is considered to fulfil the definition of claim 19. Therefore, claim 19 lacks novelty in the sense of article 33(2) PCT.

3.4 The progeny of claim 22 will not necessarily comprise the transgene. Therefore, the subject-matter of claim 22 cannot be considered as novel over well-known Castanea sativa plants (article 33(2) PCT).

**Invention II : cystatin**

1. The present application refers to a nucleic acid encoding a Castanea sativa cystatin (CsC) (nucleic acid : SEQ ID NO:3; polypeptide : SEQ ID NO:4), a chimeric gene comprising one or more such nucleic acids, an expression cassette, an expression vector, a plant genome or a host cell comprising such a chimeric gene, a genetically

modified plant containing such a chimeric gene stably integrated in its genome and the progeny, fruits or seeds of such plant. The application also refers to methods of improving the defense of a plant comprising the introduction of an expression cassette according to the present application into the plant.

2. Reference is made to the following documents :

D3: PERNAS MONICA ET AL: "A chestnut seed cystatin differentially effective against cysteine proteinases from closely related pests" PLANT MOLECULAR BIOLOGY, vol. 38, no. 6, December 1998 (1998-12), pages 1235-1242.

3. **Lack of novelty; article 33(2) PCT.**

3.1 The document D1 discloses a Castanea sativa cystatin having 99% identity in 102 AAS overlap with the sequence shown in SEQ ID NO:4 of the present application and the corresponding nucleic acid presents 99,4% identity in 318 nucleotides overlap with SEQ ID NO:3 of the present application. This protein is presented as a chestnut seed cystatin. The role of this protein in the protection against insects and nematodes is disclosed (p. 1235, Abstract; p. 1235, right-hand column, lines 3-14). The protein has been expressed in bacteria (p. 1236 - Bacterial expression and purification of recombinant cystatin).

Therefore, the subject-matter of claims 1, 14-18 and 20 cannot be considered as novel over the teaching of D3 (article 33(2) PCT).

3.2 Since there is no clear definition of what a "chimeric gene" should be, each cell naturally expressing a cystatin is considered to fulfil the definition of claim 19 and each seed or fruit of a plant comprising such cells is considered to fulfil the definition of claim 23. Therefore, claims 19 and 23 lack novelty in the sense of article 33(2) PCT.

3.3 The progeny of claim 22 will not necessarily comprise the transgene. Therefore, the subject-matter of claim 22 cannot be considered as novel over well-known Castanea sativa plants.

4. **Lack of inventive step; article 33(3) PCT.**

The most relevant document for assessing the inventive step of the claims is the document D3 (see point 3 above for the content)

In the light of this document, the problem to be solved by the present application can be seen as the provision of a further Castanea sativa cystatinand nucleic acid encoding it.

The application solves this problem by the provision of the protein shown in SEQ ID NO:4 and the corresponding nucleic acid shown in SEQ ID NO:3.

In order to be considered as inventive, the selection of the protein disclosed in SEQ ID NO:4 should be motivated by a technical purpose, i.e. a hitherto unknown or unexpected effect due to the choice of the specific cystatin of the present application. For the moment, the ISA fails to see such an effect for the selection of the protein of the present application, especially in the light of the fact that the protein of the present application only differs from the protein disclosed in the prior art by the addition of 3 amino acid residues at the N-terminus.

Therefore, the ISA is the opinion that the subject-matter of claims 1, 5-7 and 14-23 cannot be considered as inventive over the teaching of D3 (article 33(3) PCT)

Moreover, the fact that cystatins could be useful in the defence against fungal infection was also well-known in the art. Therefore, the ISA is the opinion that the skilled person would have contemplated using the non-inventive cystatin for treating fungus infection. Therefore, claims 24-27 cannot be considered as inventive in the sense of article 33(3) PCT.

**Invention III : β-1,3-glucanase.**

1. The present application refers to a nucleic acid encoding a Castanea sativa β-1,3-

glucanase (nucleic acid : SEQ ID NO:5; polypeptide : SEQ ID NO:6), a chimeric gene comprising one or more such nucleic acids, an expression cassette, an expression vector, a plant genome or a host cell comprising such a chimeric gene, a genetically modified plant containing such a chimeric gene stably integrated in its genome and the progeny, fruits or seeds of such plant. The application also refers to methods of improving the defense of a plant comprising the introduction of an expression cassette according to the present application into the plant.

2. Reference is made to the following documents :

D4: CHYE M-L ET AL: "BETA-1,3-GLUCANASE IS HIGHLY-EXPRESSED IN LATICIFERS OF HEVEA BRASILIENSIS" PLANT MOLECULAR BIOLOGY, NIJHOFF PUBLISHERS, DORDRECHT, NL, vol. 29, no. 2, 1995, pages 397-402.

D5: SCHAFLEITNER R ET AL: "Effect of virulent and hypovirulent Cryphonectria parasitica (Murr.) Barr on the intercellular pathogen related proteins and on total protein pattern of chestnut (Castanea sativa Mill.)" PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, vol. 51, no. 5, November 1997 (1997-11), pages 323-332.

3. **Lack of novelty; article 33(2) PCT.**

3.1 The document D4 discloses a Hevea brasiliensis β-1,3-glucanase. Said protein presents 76,7% identity with the protein shown in SEQ ID NO:6 in 309 AAS overlap. The corresponding nucleic acid presents 78,3% identity with the nucleic acid sequence shown in SEQ ID NO:5 in 930 nucleotides overlap. The implication of β-1,3-glucanases in plant defence is disclosed (p. 397, left-hand column, lines 5-12). The nucleic acid encoding the hevea brasiliensis protein was isolated by screening a cDNA library using a heterologous cDNA encoding a β-1,3-glucanase from Nicotiana plumbaginifolia (p. 398, left-hand column, line 12 to right-hand column, line 17).

Therefore and due to the clarity problem mentioned in point VIII-1 below, the subject-matter of claims 1 and 14-15 cannot be considered as novel in the

sense of article 33(2) PCT.

3.2 Since there is no clear definition of what a "chimeric gene" should be, each cell naturally expressing a β-1,3-glucanase is considered to fulfil the definition of claim 19 and each seed or fruit of a plant comprising such cells is considered to fulfil the definition of claim 23. Therefore, claims 19 and 23 lack novelty in the sense of article 33(2) PCT.

3.3 The progeny of claim 22 will not necessarily comprise the transgene. Therefore, the subject-matter of claim 22 cannot be considered as novel over well-known Castanea sativa plants (article 33(2) PCT).

## 4. Lack of inventive step; article 33(3) PCT.

The document D4 is considered as the most relevant document for the evaluation of the inventive step of the claims (see point 3 above for the content).

In the light of this document, the problem to be solved by the present application can be seen as the provision of a β-1,3-glucanase in a further plant.

The application solves this problem by the provision of a Castanea sativa β-1,3-glucanase.

The document D5 discloses the induction of β-1,3-glucanases in Castanea sativa after infection with a pathogen.

Therefore, the ISA is the opinion that the skilled person would have needed no inventive activity to contemplate isolating a nucleic acid encoding a β-1,3-glucanase, using a heterologous probe as disclosed in D4. The cloning of such a sequence in a vector, the transformation of a host cell or a plant with such a polypeptide or the use of such polypeptide for the protection of a plant against a pathogen can also not be considered as inventive.

Therefore, claims 1, 8-10 and 14-23 cannot be considered as inventive in the sense of article 33(3) PCT.

Moreover, the fact that β-1,3-glucanases could be useful in the defence against fungal infection was also well-known in the art. Therefore, the ISA is the opinion that the skilled person would have contemplated using the non-inventive β-1,3-glucanase protein for treating fungus infection. Therefore, claims 24-27 cannot be considered as inventive in the sense of article 33(3) PCT.

**Invention IV : Thaumatin-like protein.**

1. The present application refers to a nucleic acid encoding a Castanea sativa Thaumatin-like protein (nucleic acid : SEQ ID NO:7; polypeptide : SEQ ID NO:8), a chimeric gene comprising one or more such nucleic acids, an expression cassette, an expression vector, a plant genome or a host cell comprising such a chimeric gene, a genetically modified plant containing such a chimeric gene stably integrated in its genome and the progeny, fruits or seeds of such plant. The application also refers to methods of improving the defense of a plant comprising the introduction of an expression cassette according to the present application into the plant.

2. Reference is made to the following document :

D6: GARCIA-CASADO GLORIA ET AL: "Characterization of an apoplastic basic thaumatin-like protein from recalcitrant chestnut seeds" PHYSIOLOGIA PLANTARUM, vol. 110, no. 2, October 2000 (2000-10), pages 172-180.

3. **Lack of novelty; article 33(2) PCT.**

3.1 The document D6 discloses an apoplastic basic thaumatin-like protein from recalcitrant chestnut seeds (Castanea sativa). The protein disclosed in D6 presents 93,5% identity in 62 AAS overlap with the protein of the present application. The corresponding nucleic acid presents 99,4% identity in 732 nucleotides with the nucleic acid shown in SEQ ID NO:7. The nucleic acid has

been cloned in a vector (lambda Uni-ZAP XR) which has been used to transform E. coli SOLR cells (p. 173, cDNA cloning). The antifungal activity of the thaumatin-like protein is also disclosed (p. 172. right-hand column, lines 7-10).

Therefore, the subject-matter of claims 1, 14-18 and 20 cannot be considered as novel in the sense of article 33(2) PCT.

3.2 Since there is no clear definition of what a "chimeric gene" should be, each cell naturally expressing a thaumatin-like protein is considered to fulfil the definition of claim 19 and each seed or fruit of a plant comprising such cells is considered to fulfil the definition of claim 23. Therefore, claims 19 and 23 lack novelty in the sense of article 33(2) PCT.

3.3 The progeny of claim 22 will not necessarily comprise the transgene. Therefore, the subject-matter of claim 22 cannot be considered as novel over well-known Castanea sativa plants (article 33(2) PCT).

## 4. Lack of inventive step; article 33(3) PCT.

D6 is considered as the most relevant document for evaluating the inventive step of the claims of the present application (see point 3 above for the content of said document).

In the light of the teaching of D6, the problem to be solved by the present application can be considered as the provision of a further Castanea sativa thaumatin-like protein.

The application solves this problem by the provision of the protein shown in SEQ ID NO:8 and the corresponding nucleic acid (SEQ ID NO:7).

The ISA is the opinion that the skilled person would have needed no inventive activity to contemplate using the nucleic acid disclosed in D6 in order to isolate further

thaumatin-like protein encoding nucleic acids in Castanea sativa. Thus, in order to be considered as inventive, the selection of the polypeptide of the present application should be motivated by a technical purpose, i.e. a hitherto unknown or unexpected effect due to the selection of the specific polypeptide of the present application over the polypeptide disclosed in D6. For the moment, the ISA fails to see such an effect for the selection of the polypeptide of the application, The attention of the applicant is also drawn to the fact that the southern disclosed in D6 (Fig. 4) suggests the presence of more than one thaumatin-like gene in Castanea sativa.

Therefore, the subject-matter of claims 1, 11-23 cannot be considered as inventive in the sense of article 33(3) PCT.

Moreover, the fact that thaumatin-like proteins could be useful in the defence against fungal infection was also well-known in the art. Therefore, the search authority is the opinion that the skilled person would have contemplated using the non-inventive thaumatin-like protein for treating fungus infection. Therefore, claims 24-27 cannot be considered as inventive in the sense of article 33(3) PCT.

## Re Item VIII

## Certain observations on the international application

**Inventions I, II, III and IV.**

1. In claim 1, the nucleic acids are characterized by reference to their origin (Castanea sativa Mill.). The attention of the applicant is drawn to the fact that, once isolated, the only way to determine the origin of a nucleic acid is by reference to its specific nucleic acid or amino acid sequences. Therefore, the origin of the nucleic acids cannot be considered as a valid technical feature for the characterization of the nucleic acids of claim 1.

2. The attention of the applicant is drawn to the fact that the nucleic acids of claim 1 are not characterized by any technical feature and thus, claim 1 lacks clarity (article 6 PCT).

3. The subject-matter of claim 15 seems to be redundant with the subject-matter of claim 1 since the fact that the nucleic acid "can be used together with other genes expressed in Castanea sativa Mill." does not appear to imply any further technical feature of the nucleic acid.

4. The attention of the applicant is drawn to the fact that the wording "chimeric" does not imply any technical characteristic per se. Therefore, each gene encoding an allene oxide synthase, a cystatin, a β-1,3-glucanase or a thaumatin-like protein is considered to fit the definition of claim 16.

5. Due to the problem mentioned in point 4 above, the genome of each cell naturally expressing a allene oxide cyclase, a cystatin, a β-1,3-glucanase or a thaumatin-like protein is considered to fulfil the definition of claim 19.

6. The progeny of claim 22 will not necessarily comprise the transgene. Therefore, the subject-matter of claim 22 encompasses normal plants.